FULL ARTICLE

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Epidemiological characteristics of *Leptospira* spp. infection in bovine herds in Mato Grosso do Sul, Brazil*

Caracterização soroepidemiológica da infecção por Leptospira spp. em rebanhos bovinos de corte do estado de Mato Grosso do Sul, Brasil

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ABSTRACT

The epidemiological characteristics of bovine leptospirosis in animals and herds in Mato Grosso do Sul were investigated to determine parameters such as disease frequency and the serovars reactant in beef cattle herds. A total of 4,629 beef cattle herds were examined against 33 *Leptospira spp.* serovars. The serum samples were submitted to the microscopic agglutination test (MAT) for the serological diagnosis of leptospirosis. The MAT results showed that 3,814 (82.39%) of the 4,629 animals evaluated were seropositive for the bacterium, with serological reactions mainly to serogroup Sejroe, serovar Wolffi (36.49%). The observed high frequency of reactive animals demonstrates the relevance of the infection. Therefore, general and specific measures should be implemented to contain and/or prevent infection of the animals in the studied region.

Keywords: Leptospirosis. Beef cattle. Microscopic Agglutination Test (MAT). Brazilian strains.

RESUMO

Foi realizado um inquérito epidemiológico da leptospirose em bovinos de rebanhos de corte do estado de Mato Grosso do Sul, de modo a determinar a frequência e as sorovariedades reagentes. Para isso, foram examinados 4.629 bovinos de corte, com uma coleção de 33 sorovariedades de *Leptospira,* por meio da prova de Soroaglutinação Microscópica (MAT). Dos 4.629 animais examinados, 3.814 (82,39%) foram reagentes com reações predominates para o sorogrupo Sejroe, sorovar Wolffi (36,49%). Assim, a alta frequência de animais reagentes encontrada justifica a implantação de medidas gerais e específicas para conter e/ou prevenir a infecção nos animais dessa região.

Palavras-chave: Leptospirose. Bovinocultura de corte. Soroaglutinação Microscópica (MAT). Estirpes brasileiras.

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Introduction

Beef cattle play a particularly important role in the Brazilian economy. Brazil has the largest commercial herd and is the largest beef exporter (Instituto Brasileiro de Geografia e Estatística, 2015) in the world. Thus, Brazil is under continuous pressure for increased productivity and profit, which can cause greater agglomerations and more favorable epidemiological conditions for maintaining and transmitting pathogens such as *Leptospira* spp. in the herds.

In cattle, leptospirosis is mainly linked with reproductive problems such as abortions and the birth of stillborn and/or weak animals (Oliveira et al., 2010), which causes economic losses due to dropping meat and milk production, as well as additional costs with veterinary assistance, vaccination and laboratory tests (Faine, 1993).

The Hardjo, Wolffi, Pomona, Grippotyphosa, and Icterohaemorrhagiae serovars are highlighted in the bovine species. The Hardjo and Wolffi serovars belong to the same serogroup and are described as the most frequent in the bovine species. However, in Brazil, although the Wolffi serovar is frequently detected by serology, it was never isolated in cattle.

Besides confirming the infection diagnosis, isolating *Leptospira* spp. in a herd with infected cattle is certainly an important step for implementing disease control programs, since it allows including native serovars in vaccines and the diagnostic microscopic agglutination test, MAT (Castro et al., 2008; Sarmento et al., 2012).

Thus, considering the cattle herd in Mato Grosso do Sul and the reproductive losses that may occur due to infection by *Leptospira* spp., it is important to determine the occurrence and spatial distribution of the disease in this state herds. These data should allow the adoption of prevention and control measures against the predominant serovars.

Thus, the objective of this study was to investigate the epidemiological characteristics of bovine leptospirosis in the animals and herds in one of the most important cattle ranching states, Mato Grosso do Sul, MS, in Brazil.

Materials and Methods

The study was approved by the Ethics Committee on Animal Use (CEUA), protocol 016626/14. A total of 4,629 blood serum samples were collected from male and female beef cattle (Pure-Bred Nellore, Commercial Nellore, Senepol, Caracu, and Crossbred) of different ages, from 10 herds (Table 1). The herds were chosen for convenience and extensive rearing system, in Mato Grosso do Sul. The samples analyzed in this study were collected in 2014 by EMBRAPA Gado de Corte in Campo Grande, MS, for research on leptospirosis and other diseases of interest

Table 1 – Herds (beef cattle) and their respective municipalities, mesoregions, breed, and the number of collected blood samples (nº), Mato Grosso do Sul, Brazil, 2014

Herd	City	Mesoregion	Breed	Vac*	n°
1	Rio Brilhante	Southwest	Pure-bred Nellore /Crossbread	No	126
2	Campo Grande	North central	Commercial Nellore/ Caracu/Senepol/ Crossbread	No	501
3	Campo Grande	North central	Pure-bred Nellore / Crossbread	Yes	535
4	Terenos	North central	Pure-bred Nellore / Commercial Nellore	No	694
5	Brasilândia	East	Commercial Nellore/ Crossbread/ Senepol	No	639
6	Dois Irmãos do Buriti	Pantanais	Pure-bred Nellore	Yes	419
7	Miranda	Pantanais	Pure-bred Nellore	Yes	522
8	Rio Verde de Mato Grosso	North central	Pure-bred Nellore /Commercial Nellore / Crossbread	Yes	526
9	Camapuã	North central	Senepol/ Crossbread	No	445
10	Miranda	Pantanais	Pure-bred Nellore	**	222
Total					4.629

* Vaccination;**Some animals in the herd are vaccinated.

in cattle herds in this region and were kindly provided for this study.

The collected samples were sent to the Virology Laboratory (EMBRAPA Gado de Corte) and stored at -20 °C until the serological tests. The microscopic agglutination test (MAT) was used to diagnose leptospirosis.

The blood serum samples were initially diluted in saline solution at 1/50. Aliquots of 25 μL were placed on flat-bottom polystyrene plates with an added equal amount of antigen from the 33 serovars of *Leptospira* spp. The used strains/serovars previously isolated in Brazil such as Guaicurus, Goiano, LO10, LO04, GR6, 2ACAP, Brasiliensis, 110/06, and LO14 were provided by the Leptospirosis and Brucellosis Laboratory (UNESP – Jaboticabal). The reference strains used were Andamana, Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Sentot, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes, Hardjo, Patoc, Wolffi, Shermani and Tarassovi.

The serum-antigen mixture was lightly homogenized and incubated in a BOD oven at 28 °C for 40 to 120 min, followed by reading in dark field microscopy, with a 10x objective and eyepiece, directly from the plate wells. Samples with a titer ≥ 100 were considered positive. The plate readings were performed by the same individual to minimize any interpretation bias. The samples reactive in the initial dilution were tested with serial dilutions from 1/100 to 1/800 at ratio two, following the recommendation of the World Organisation for Animal Health (2001).

To determine the frequency of occurrence, animals reactive to one or more serovars were considered seropositive. Only the serovar with the highest titer was considered as the most probable serovar while samples that presented equal titer against two or more serovars were disregarded.

The statistical analysis was performed to determine frequency distribution and calculate the confidence interval of prevalence rates per herd, using the methodology recommended by Thrusfield (2010). The difference between the frequencies of positive animals was analyzed according to the most likely source of infection serovar, and calculated based on the difference between the confidence intervals. The study was carried out using MapInfo®, and the density of cases was evaluated with kernel density analysis to determine hotspots.

Results

Of the 4,629 animals tested by the MAT, 3,814 (82.39%) were seropositive for at least one of the 33 serovars used for *Leptospira* spp., with a titer equal or above 100 to one or more leptospiral serovar. The frequency of occurrence of herds with positive animals was 100%, considering reactions to any of the serovars used as an antigen. Herd 7 in Miranda had the highest number of positive animals, 98.66% (515/522), whereas herd 3 in Campo Grande had the lowest, 65.61% (351/535) (Table 2).

The results showed that all eight municipalities (100%) had animals positive to one or more of the tested leptospiral serovars (Table 3). Miranda had the highest number of positive animals, with 92.60% (689/744) whereas Camapuã had the lowest 71.91% (320/445) (Figure 1).

Of the 33 antigens used, reactions to Wolffi serovar were the most frequent 36.49% (905/2,480), followed by Shermani, 18.43% (457/2,480), and Hebdomadis, 8.66% (215/2,480). Among the nine strains isolated in Brazil, the highest occurrence of reaction was observed for Guaicurus,

Table 2 – Frequency (number and %) cattle reactive at least to one *Leptospira* spp. serovar in the microscopic agglutination test (MAT) using 33 antigens, according to herd, Mato Grosso do Sul, Brazil, 2014

Herd	MAT		CI 95% (%)
	Reactive*	(%)	
1	91/126	72.22	64.40-80.04
2	430/501	85.83	82.77-88.88
3	351/535	65.61	61.58-69.63
4	531/694	76.51	73.36-79.67
5	559/639	87.48	84.91-90.05
6	365/419	87.11	83.90-90.32
7	515/522	98.66	97.26-99.35
8	478/526	90.87	88.41-93.34
9	320/445	71.91	67.73-76.09
10	174/222	78.38	72.96-83.79
Total	3.814/4.629	82.39	81.30-83.49

*Positive animals/total examined animals.

Table 3 – Frequency (number and %) of blood serum samples from cattle reactive to at least one *Leptospira* spp. serovar in the microscopic agglutination test (MAT) using 33 antigens, according to the city in Mato Grosso do Sul State, Brazil, 2014

City	MAT		CI 95% (%)
	Reactive*	(%)	
Brasilândia	559/639	87.48	84.91-90.05
Camapuã	320/445	71.91	67.73-76.09
Campo Grande	781/1.036	75.39	72.76-78.01
Dois Irmãos do Buriti	365/419	87.11	83.90-90.32
Miranda	689/744	92.60	90.73-94.49
Rio Brilhante	91/126	72.22	64.40-80.04
Rio Verde de M. Grosso	478/526	90.87	88.41-93.34
Terenos	531/694	76.51	73.36-79.67
Total	3.814/4.629	82.39	81.30-83.49

*Positive animals/total examined animals.

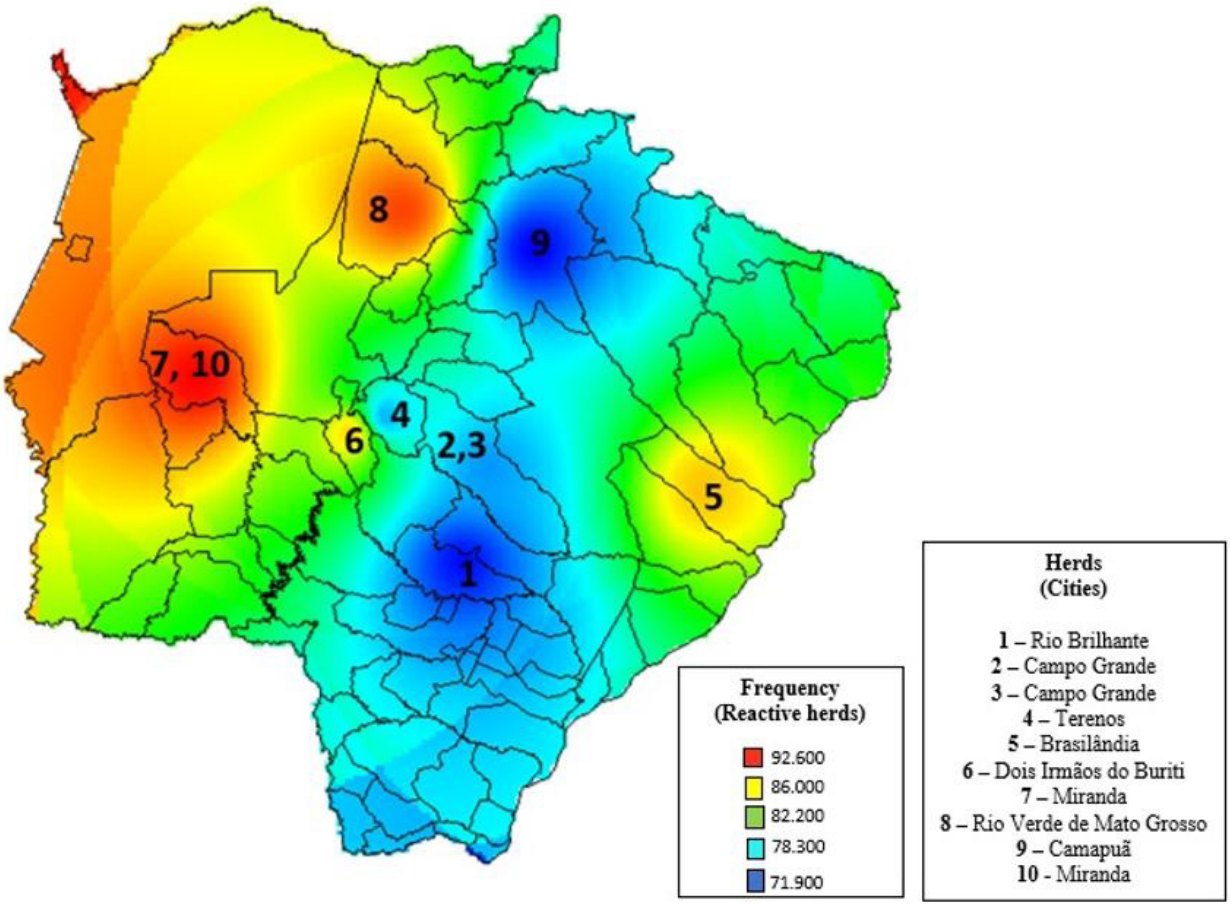

Figure 1 – Frequency (%) of blood serum samples from cattle reactive/positive to at least one *Leptospira* spp. serovar in the Microscopic Agglutination Test (MAT) using 33 leptospiral serovars in cities in Mato Grosso do Sul State, Brazil, 2014.

with a frequency of 2.5% (62/2,480), followed by Goiano, 1.25% (31/2,480), and LO10 0.77% (19/2,480).

Reaction to Wolffi serovar was found in eight of the 10 herds analyzed. It was also the most prevalent, except for one of the herds, in which it was the second-highest occurrence.

Regarding the probable serogroup causing the infection, the most frequent was Sejroe (Hardjo, Wolffi and Guaricura strains), with a frequency of 39.96% (991/2,480), followed by Shermani (Shermani strain), 18.43% (457/2,480), and Hebdomadis (Goiano and Hebdomadis strains), 9.92% (246/2,480).

Discussion

The frequency of serologic reactions of 82.39% (95% CI: 81.30% - 83.49%) animals seropositive to *Leptospira* spp. in MAT and, 100% of herds reactive, was considerably high. Figueiredo et al. (2009) also reported a high prevalence, 98.8% (1,801/2,573), of cattle reactive to *Leptospira* spp. in Mato Grosso do Sul. According to Oliveira et al. (2010), seroreactivity fluctuations over time can be attributed to intrinsic and extrinsic factors that modify the epidemiological triad (etiologic agent, host, and environment).

Faine (1982) concluded that, compared to temperate regions, disease presence and dispersion are more favored in tropical and subtropical regions due to persistent bacteria in flooded environments, where they can survive for up to 180 days, depending on temperature (28 °C to 30 °C) and pH (7.2 to 7.4) conditions. In Mato Grosso do Sul, the tropical climate prevails in most of the territory, characterized by rainy summers and dry winters, with average temperatures varying between 26°C in the Paraguay lowlands and 23°C in the Plateau, and approximately 1,500 mm rainfall annually (Borlachenco & Gonçalves, 2017).

This high frequency of animals seropositive to *Leptospira* can also be attributed to the cattle breeding system adopted in Mato Grosso do Sul. The extensive rearing system widely used for the cattle herds favors the occurrence of the disease since the cattle herds are in contact with other animal species, especially wild ones, which can be important reservoirs of the disease etiological agent, favoring the permanence and spread of leptospira in the herd.

The spatial distribution of seropositive animals per herd shows that the highest number was observed in herd seven, in Miranda, the Pantanais mesoregion. Vieira (2009) stated that the ecological and climatic conditions in the Pantanal Sul-Matogrossense are highly favorable to the occurrence of leptospirosis since the etiological agent survives longer in flooded areas and high temperatures.

The occurrence of animals positive to Wolffi (36.49%), Shermani (18.43%), and Hebdomadis (8.66%) serovars was predominant in this study. In contrast, most recent serological surveys demonstrate that Hardjo serovar is the most likely to infect cattle (Coelho et al., 2014; Juliano et al., 2016; Pimenta et al., 2014).

Because the Wolffi serovar was the probable cause of infection, and there were reports of abortions in all herds, it is suggested that this serovar plays an important role as a cause of reproductive losses. While many studies report Hardjo and Wolffi as an associated occurrence, in this study the Wolffi and Shermani serovars were the predominantly associated occurrence, detected in 50% of the analyzed herds. The Wolffi predominance reinforces the importance of research to isolate this serovar since it has never been isolated from Brazilian cattle and may be important in the dissemination and maintenance of leptospirosis in Brazilian herds.

Also, it should be emphasized that the Shermani serovar has already been isolated from rodents in Brazil (Lins & Santa Rosa, 1976), and this Brazilian isolate was obtained in Mato Grosso, a state that borders a large part of Mato Grosso do Sul territorial extension. It is noteworthy that when collecting the samples for this study, the producers/employees informed about the presence of rodents on the farms, especially capybaras (*Hydrochoerus hydrochaeris*), which are wild rodents, and also peccary (*Tayassu tajacu*). Ahmed et al. (2006) and Marvulo et al. (2002) have already isolated leptospires from the kidney of slaughtered capybaras while several studies have recorded the occurrence of capybaras seropositive to *Leptospira*, thus confirming the important role of this species as a bacterium reservoir.

Regarding the serovars that most likely have infected the herds in Mato Grosso do Sul, it is noteworthy that the acquired immunity is serovar-specific, and immunization protects only against homologous or antigenically similar serovars. The commercial vaccines for cattle available on the market have in their composition the antigens: Pomona, Wolffi, Hardjo, Icterohaemorrhagiae, Canicola, and Grippotyphosa, which do not protect against the serovars Shermani and Hebdomadis that were highly prevalent in this and other studies on bovine leptospirosis. In addition to vaccination, Castro et al. (2008) also pointed out that the serovars Shermani, Hebdomadis, and Autumnalis are often not present in routine tests and, although they may be infecting herds, they are not being detected.

Prophylactic measures should be adopted to reduce the prevalence of infection by serovars present the population since the etiologic agent is responsible for low reproductive performance caused by infertility, abortions, stillbirths, premature births, and the birth of weak calves, among others, which consequently represent economic losses for beef cattle.

In general, the high prevalence of leptospirosis in animals and herds is emphasized, requiring the adoption of general and specific measures for preventing and controlling this disease, to improve the animal production indicators of the beef herds in the region.

Conclusions

The high frequency of reactive animals shows that infection with *Leptospira* spp. is occurring in herds studied in Mato Grosso do Sul, demonstrating the relevance of infection by the etiological agent. The animals were reactive to different serovars, with Wolffi serovar the most likely to infect the animals of the herds in Mato Grosso do Sul. Special attention should be paid to the Guaicurus serovar due to its significant occurrence in the analyzed herds. Thus, we suggest that it be inserted in the antigen collection when animals from the region are analyzed by the microscopic agglutination test.

Conflict of Interest

The authors declare no conflicts of interest.

Ethics Statement

Protocol 016626/14 Ethics Committee on Animal Use (CEUA) – Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal.

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